A simple single-step procedure for small-scale preparation of *Escherichia coli* plasmids

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Numerous rapid procedures to prepare relatively pure plasmid DNA from small volumes of *E. coli* cultures have been developed for restriction mapping purposes [1–4]. Some of these procedures demand lengthy fractionation steps involving lysozyme-mediated cell lysis, boiling, phenol/chloroform extractions and DNA precipitations. Here we describe a procedure which allows isolation of plasmid DNA on a mini-scale, demanding no more than 20 minutes. The principle is based on the finding that phenol/chloroform treatment of *E. coli* cells in the presence of LiCl and Triton X-100 solubilises the plasmid DNA, concomitantly precipitating the unwanted denatured chromosomal DNA and the cellular proteins. The procedure is as follows:

A 1.5 ml *E. coli* culture carrying the plasmid of interest was grown in Luria broth (LB) with 100 μg/ml ampicillin for 16 h. Alternatively, a 2 mm diameter *E. coli* transformant colony grown on LB/agar plate was scraped and suspended into 1.5 ml of 10 mM Tris-HCl (pH 8.0), 1 mM Na_2EDTA (TE). The cells were harvested in an Eppendorf tube by centrifugation at 10,000×g for 0.5 min and suspended in 100 μl of 2.5 M LiCl, 50 mM Tris-HCl (pH 8.0), 4% (v/v) Triton X-100, 62.5 mM Na_2EDTA, followed by addition of an equal volume of phenol/chloroform mixture (1:1 (w/v)). The mixture was vigorously vortexed for 15 seconds and centrifuged for 1 min at 15,000×g at 22°C. The upper phase of nucleic acids was collected, mixed with two volumes of absolute EtOH and the precipitated nucleic acids harvested by centrifugation at 15,000×g for 10 min at 4°C. The pellet was washed with 1 ml of absolute EtOH and harvested by brief centrifugation. After drying under vacuum, the DNA was dissolved in 30 μl of TE. In trials with different plasmids and different *E. coli* strains recovery of the nucleic acids ranged from 2 to 3 μg. Figure 1 shows a typical preparation of a plasmid construct used for restriction mapping. The preparations were treated with DNAase-free RNAase A (10 μg/ml) in the digestion mixture to eliminate the RNA interfering with detection of low molecular weight DNA fragments. The plasmid preparations were suitable for restriction mapping all of the following restriction enzymes tested: *Hin*dIII, *Bam*HI, *Eco*RI, *Hin*cII, *Pst*I, *Xba*II and *Sma*I. Restricted fragments were suitable substrates in ligation reactions. The procedure also works well for large-scale preparation (from 5 to 50 ml cultures) of plasmids. The plasmid fractionated immediately after TELT/phenol/chloroform was also suitable for direct transformation of competent *E. coli* cells. That such plasmids introduced into *E. coli* were intact and functional was confirmed by the ability of (i) pBR322 to impart the bacterium with resistance to ampicillin and tetracycline, and (ii) the construct pEI-W3 to express upon thermo-induction the cloned heterologous gene product, pre-SS-RUBISCO

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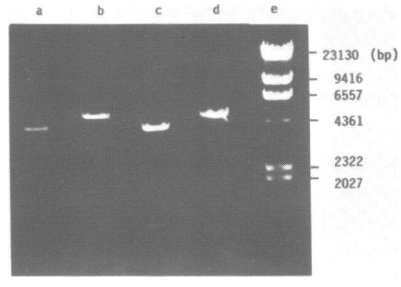

Figure 1. Analysis of the plasmid construct pEI-WPrA, carrying an insert of 300bp of protein A in pEI-W3 , in 0.8% agarose gel after restriction endonuclease digestion of 5 μl (approximately 0.25 μg DNA) of the plasmid preparation with (a) *Sma*I, (b) *Bam*HI, (c) *Eco*RI and (d) *Hin*dIII. Track (e), *Hin*dIII-digested lambda DNA fragments as markers.

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